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# Experimental mucositis and experimental gingivitis in persons aged 70 or over. Clinical and biological responses

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**Key words:** adult, aged, biomarkers, dental implants, gingival crevicular fluid, gingivitis, mucositis, oral hygiene

## Abstract

**Objectives:** To compare in persons aged 70 years or older the clinical and inflammatory changes occurring around implants and natural teeth during and after a phase of undisturbed plaque accumulation.

**Material and methods:** Twenty partially edentulous participants with titanium implants refrained from oral hygiene practices while being clinically monitored in weekly intervals for 21 days. Teeth and implants were then cleaned, oral hygiene resumed, and the participants were further monitored for 3 weeks. Twelve biomarkers were assessed in gingival and peri-implant crevicular fluid (GCF, PCF).

**Results:** During 3 weeks of oral hygiene abstention, the gingival index (GI) continuously increased. On day 21, there were significantly more sites with GI >1 at implants than at teeth. After restarting oral hygiene, the GI decreased markedly in both groups. Throughout the experiment, the plaque index was significantly higher on teeth than on implants. The different biomarkers reacted variably. IL-1 $\beta$  increased significantly with plaque accumulation. IL-1 $\beta$ , GM-CSF, TNF- $\alpha$ , and IFN- $\gamma$  were significantly higher in GCF compared to PCF at day 21. IL-8 decreased significantly in GCF up to day 14. MIP-1 $\beta$  decreased significantly in GCF, but not in PCF. At the 3-week follow-up, the levels of all biomarkers assessed in GCF and PCF had returned to baseline values.

**Conclusions:** In an elderly cohort, plaque accumulation induced an inflammatory reaction around both teeth and implants. Although there was less plaque accumulation on implants, the peri-implant mucosa showed a stronger clinical response than gingiva.

In 1965, a groundbreaking publication (Löe et al. 1965) demonstrated for the first time convincingly that the accumulation of bacterial deposits on teeth can cause inflammation in the gingiva. Twelve young volunteers with relatively healthy gingiva were asked not to clean their teeth anymore. In the absence of oral hygiene, bacterial deposits formed on the teeth, initially detectable only after moving a dental instrument on the tooth surface, later recognizable with the naked eye as a layer of soft matter. Within 9–21 days, the gingiva started to show signs of inflammation such as redness, change in texture, and bleeding on pressure. Once the inflammation was established, detailed instructions were given to the participants to clean the teeth again properly. As a consequence of the bacterial deposits being removed, the inflammation of the gingiva disappeared. Other investigators have repeated this experiment in diverse

human cohorts and with various modifications, corroborating the general association between poor oral hygiene and inflammation (Theilade 1996). The effect of plaque on implants on the peri-implant mucosa has also been studied. Reports indicated that accumulation of bacterial deposits on implants induced inflammation in the peri-implant mucosa in a similar way as dental plaque produced gingivitis (Pontoriero et al. 1994; Zitzmann et al. 2001). A more recent study concluded that the inflammatory response seemed to be even stronger in the peri-implant mucosa than in the gingiva (Salvi et al. 2012). Heterogeneity in the development of clinical signs of gingivitis had already been noted by Löe et al. and was initially thought to be due to differences in either the plaque volume or the microbial composition (Theilade et al. 1966). Today, it is recognized that the gingival inflammation

**Date:**  
 Accepted 18 May 2016

## To cite this article:

Meyer S, Giannopoulou C, Courvoisier D, Schimmel M, Müller F, Mombelli A. Experimental mucositis and experimental gingivitis in persons aged 70 or over. *Clinical and biological responses*. *Clin. Oral Impl. Res.* 28, 2017, 1005–1012. doi: 10.1111/clr.12912

may also depend on host factors modulating the inflammatory response to the presence of bacteria (Hajishengallis & Lamont 2012).

Good oral hygiene is not a common finding among elderly people (Kay & Locker 1996). Even though the prevalence of gingivitis varied among the different populations studied, it was suggested that the majority of the elderly patients had a tendency for a more severely inflamed gingiva (MacEntee 2005). Following the original report of a cause-and-effect relationship between dental plaque and gingivitis, the effect of age on the development of experimental gingivitis was evaluated (Fransson et al. 1996). Although the elderly participants showed similar amounts of plaque as the young subjects, they developed more gingivitis. Substantial age-associated abnormalities in the immune cell-response, that is, neutrophils, macrophages, dendritic cells, and Langerhans cells, may contribute to the increased susceptibility of the aged individuals to periodontal disease (Zavala & Cavicchia 2006).

The analysis of the gingival crevicular fluid (GCF) composition is a non-invasive method to assess the inflammatory conditions of the periodontal tissues (Cimasoni 1983). In analogy, various molecules have been analyzed in the peri-implant crevicular fluid (PCF). Levels of several biomarkers differed with regard to the clinical status of the gingiva and peri-implant mucosa (Plagnat et al. 2002; Nogueira-Filho et al. 2014; Hall et al. 2015; Recker et al. 2015).

Only few studies have assessed the influence of plaque accumulation on clinical parameters and host-derived factors in elderly individuals. Therefore, the aim of this study was to compare in persons aged 70 years or older, the clinical and inflammatory changes occurring around implants and natural teeth during a phase of undisturbed plaque accumulation and after reinstitution of mechanical plaque control.

## Material and methods

This was a single-center, three-phase experimental gingivitis/mucositis trial, with intra-patient comparison. The Ethical Committee of the University Hospitals of Geneva, Geneva, Switzerland, approved the study protocol. The study was conducted according to the principles outlined in the Declaration of Helsinki on human medical experimentation. All participants were informed about the procedures and signed a consent form in advance of their inclusion in the study.

## Participants

Twenty-one participants were recruited between January 2014 and May 2015 from patients previously treated at the University of Geneva School of Dental Medicine. The clinical procedures and evaluations were carried out between June 2014 and July 2015.

The participants were included based on the following criteria: aged 70 years or older, partially edentulous with presence of titanium implants, in good general and oral health. We excluded patients with peri-implantitis and/or periodontitis, specifically those with periodontal or peri-implant pockets deeper than 4 mm with bleeding or pus, with major systemic illnesses (level P3 and higher according to the ASA classification; Dripps et al. 1961), specifically those with uncontrolled diabetes mellitus, cancer, bone metabolic diseases, or disorders that compromise wound healing, radiation, or immunosuppressive therapy, those with evidence for an infection in the upper respiratory, pulmonary, digestive, or renal tract in the last 2 weeks, systemic antibiotics taken within the previous 2 months, or systemic non-steroidal anti-inflammatory drugs in the previous month, and heavy smokers ( $\geq 20$  cigarettes/day).

## Clinical protocol

The study had three parts: In the first phase, bacterial deposits were removed from all teeth and implants, and detailed oral hygiene instructions were given to all participants. The ability to perform proper plaque control was assessed after one, two, and 3 weeks. If necessary, further instructions were given, and additional visits were scheduled. A full-mouth plaque score (PS, percentage of sites with plaque, four sites per tooth or implant, detected when running a probe across a site)  $< 20\%$  was required to enter the next stage. In the second phase, the participants refrained from oral hygiene practices while being monitored in weekly intervals for 3 weeks. At the beginning of the third phase, the accumulated bacterial deposits were removed and instructions were given to clean teeth and implants again properly. The participants were further monitored after one and 3 weeks. A minimum of nine visits was necessary to complete the study.

One calibrated examiner (S.M.) performed all procedures involving a contact with the participants. These included patient enrollment, tooth cleaning and oral hygiene instructions, clinical measurements, and sampling of GCF and PCF. Two implants

and two teeth were selected at the beginning of the study for longitudinal monitoring and fluid sampling. If present, the first premolar on each side was selected in the partially edentulous arch. If the first premolar was missing, the next adjacent mesial tooth was selected. The following clinical parameters were recorded: Plaque index (PI) (Silness & L  e 1964), gingival index (GI) (L  e & Silness 1963), probing depth (PD), recession (REC; positive if gingival margin located apical, negative if located coronal to the cemento-enamel junction or implant shoulder), bleeding on probing (BOP). The GI was originally defined to assess natural teeth, not implants; hence, the modified sulcus bleeding index (Mombelli et al. 1987) would have been more suitable for assessing implants. However, as implants were compared to natural teeth in this study, utilization of GI for implants was considered more appropriate. The clinical parameters were measured at six sites per unit. The assessments were made as shown in Table 1.

Samples of GCF and PCF were obtained from the mesio-vestibular and disto-lingual/palatal aspects of the study teeth and implants. Prior to sampling, the area of collection was isolated with cotton rolls. Each specific site was cleaned locally with a cotton pellet and dried with an aspiration tip. After 2 min, a  $2 \times 6$  mm strip of Durapore<sup>®</sup> membrane, pore size of  $0.22 \mu\text{m}$  (Millipore, Bedford, MA, USA), was placed at the entrance of the crevice and left for 30 s to collect the newly formed fluid. The two strips from one tooth or implant were put together into one microtube. Specimens were stored at  $-20^\circ\text{C}$  until analyzed.

## Laboratory procedures

Biomarkers in GCF and PCF were assessed using a multiplex fluorescent bead-based immunoassay and the Bio-Plex 200 suspension array system (Bio-Rad Laboratories, Hercules, CA, USA) as previously described (Cionca et al. 2016). Twelve inflammatory markers were measured: Interleukin (IL)-1 $\beta$ , IL-1 receptor antagonist (IL-1ra), IL-6, IL-8, IL-17, basic fibroblast growth factor (basic-FGF), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon- $\gamma$  (IFN- $\gamma$ ), macrophage inflammatory protein-1 $\beta$  (MIP-1 $\beta$ ), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), vascular endothelial growth factor (VEGF). The detection limit of the assay varied between 1.0 and 2.2 pg/ml, except for IL-1ra (5.6 pg/ml) and TNF- $\alpha$  (6.6 pg/ml).

**Table 1. Protocol of the study**

	Preparatory phase			Plaque accumulation				Oral hygiene	
	Visit 1 (Pre-baseline)	Visit 2 (Baseline)	Visit 3 (PS<20%)	Visit 4 (Day 0, no hygiene)	Visit 5 (Day 7, no hygiene)	Visit 6 (Day 14, no hygiene)	Visit 7 (Day 21, no hygiene)	Visit 8 (Day 28, PS<20%)	Visit 9 (Day 42, PS<20%)
Subject screening	X								
Informed consent	X								
Health history, medications	X	X	X	X	X	X	X	X	X
Professional plaque removal		X	X	X			X	X	X
Oral hygiene reinforcement		X	X				X	X	X
GCF/PCF sampling		X		X	X	X	X	X	X
PS		X	X	X				X	X
PD, BOP, REC		X		X			X		X
PI, GI		X	X	X	X	X	X	X	X

### Statistical analysis

The Bio-Plex Manager Software 3.0 (BIO-RAD, Hercules, CA, USA) was used for biochemical data acquisition and processing. A constant (0.1) was added to all readings to remove zero values. For all data recorded at teeth and implants at multiple sites (PI, GI, PD, REC, BOP), an individual average was calculated for teeth and implants for each visit. For part of the analysis, the GI scores were dichotomized into no or slight gingivitis without bleeding (scores 0 and 1) vs. marked gingivitis with bleeding (scores 2 and 3), referred to as GI >1. Differences between teeth and implants at specific visits, and longitudinal changes within each group, were analyzed with the Wilcoxon signed-rank test. The significance threshold was set at 0.05. The statistical software R (version 3.2.2, The R Foundation for Statistical Computing, Vienna, Austria) was used for the analyses.

## Results

Twenty-one persons gave informed consent and were enrolled in the study. One participant was lost after the second visit due to difficulties to travel. All other participants, 10 males and 10 females, attended all visits and completed the study according to protocol. Their mean age was  $77.0 \pm 5.7$  years (range 70–88 years). None of the participants was a current smoker. The mean number of teeth per person was  $16.2 \pm 5.9$ . The mean number of implants was  $4.1 \pm 2.4$ . All study implants except one (Axiom, Anthogyr SAS, Sallanches, France) were from the same manufacturer (Institut Straumann AG, Basel, Switzerland) and had a sandblasted and acid-etched titanium surface (SLA). They had been placed at various time points at least 1 year before the study. Twenty-one of the study

implants supported single-unit crowns, 12 implants supported fixed bridges, 7 carried a retentive anchor. Seven of the study teeth were without restoration, 24 had a composite filling, and 9 were crowned.

Table 2 shows the clinical and biological characteristics of the selected study teeth and implants at baseline. There was a significant difference between implants and teeth for mean PI, mean PD, and mean REC. Differences between readings of biomarkers were not statistically significant.

Figure 1 shows the intra-oral status of one participant after 21 days of no oral hygiene. Table 3 shows the clinical parameters before, during (days 7, 14, 21) and after plaque accumulation (days 28, 42). After 1 week without oral hygiene (day 7), implants and teeth had a significantly increased mean PI. The PI increased further to reach the highest value on day 21. After reinstituting oral hygiene, the PI decreased markedly in both groups.

Figure 2 shows the average proportion of sites with a PI score of 1, 2, and 3 around teeth and implants. Throughout the experiment, PI was significantly higher on teeth than on implants.

During 3 weeks of oral hygiene abstention, the GI continuously increased, reaching a level statistically higher than at day 0 on day 14 on both teeth and implants. On day 21, there were significantly more sites with GI >1 at implants than at teeth. After resuming oral hygiene, the GI decreased markedly in both groups. There was a tendency for a more severe inflammation around implants compared to teeth, with higher proportions of sites with GI >1. At implants, GI >1 was still higher than baseline on day 28, and significantly higher compared to teeth. Figure 3 shows the average proportion of sites with a GI score of 1, 2, and 3 around teeth and implants. At day 21 of the experiment, the GI was significantly higher at implants than

**Table 2. Clinical and biological characteristics at the selected study teeth and implants for 20 participants at baseline, expressed as median and interquartile range [IQR]**

	Teeth	Implants	P*
Mean PI	0.46 [0.15; 0.77]	0.04 [0.00; 0.33]	0.007
Mean GI	0.00 [0.00; 0.08]	0.00 [0.00; 0.08]	0.60
Mean PD, mm	2.42 [2.21; 2.54]	2.96 [2.75; 3.13]	<0.001
Mean BOP	0.08 [0.00; 0.17]	0.17 [0.08; 0.19]	0.07
Mean REC, mm	1.17 [0.81; 1.75]	0.36 [0.00; 0.83]	0.002
IL-1 $\beta$ , pg/ml	145.8 [44.5; 221.7]	89.6 [42.3; 186.6]	0.79
IL-1ra, pg/ml	47.4 [27.1; 508.9]	52.6 [25.0; 512.4]	0.89
IL-6, pg/ml	2.4 [1.4; 12.4]	1.5 [1.1; 6.9]	0.27
IL-8, pg/ml	422.4 [304.3; 681.5]	481.9 [362.3; 870.8]	0.07
IL-17, pg/ml	25.6 [18.3; 31.3]	25.4 [21.9; 38.4]	0.29
Basic-FGF, pg/ml	29.6 [26.8; 32.4]	31.6 [28.9; 38.1]	0.12
G-CSF, pg/ml	716.9 [195.8; 1136.0]	485.2 [121.5; 904.2]	0.24
GM-CSF, pg/ml	43.9 [31.2; 49.1]	47.3 [30.0; 49.8]	0.52
IFN- $\gamma$ , pg/ml	118.3 [86.6; 144.4]	127.2 [94.5; 165.3]	0.20
MIP-1 $\beta$ , pg/ml	13.4 [7.3; 26.8]	19.8 [10.9; 42.1]	0.06
TNF- $\alpha$ , pg/ml	13.0 [7.3; 19.9]	12.2 [9.1; 22.6]	0.07
VEGF, pg/ml	338.6 [231.5; 636.4]	422.5 [334.3; 593.4]	0.30

\*Difference between groups.



Fig. 1. Clinical status of one participant after 21 days without oral hygiene, showing plaque accumulation and gingivitis.

teeth. There was a high variability in the proportion of sites with GI >1 among participants, from day 0 to day 42, as can be seen in Fig. 4.

PD and REC were significantly higher for implants than for teeth throughout the study. PD was slightly but significantly increased in both groups on day 21. BOP increased significantly in both groups with plaque accumulation, more so around implants than around teeth. At day 42, the BOP had returned to scores comparable to baseline.

#### Host-derived parameters

Table 4 shows the concentrations of biomarkers, expressed in pg/ml before, during and after plaque accumulation. At day 0, IL-6 was the only biomarker to be significantly more concentrated in GCF compared to PCF. During the plaque accumulation phase, the different biomarkers reacted variably. IL-1 $\beta$  showed a significantly higher concentration in both GCF and PCF on days 7, 14, and 21 compared to baseline. Furthermore, GCF concentrations were at each time point significantly higher than PCF. TNF- $\alpha$  and IFN- $\gamma$

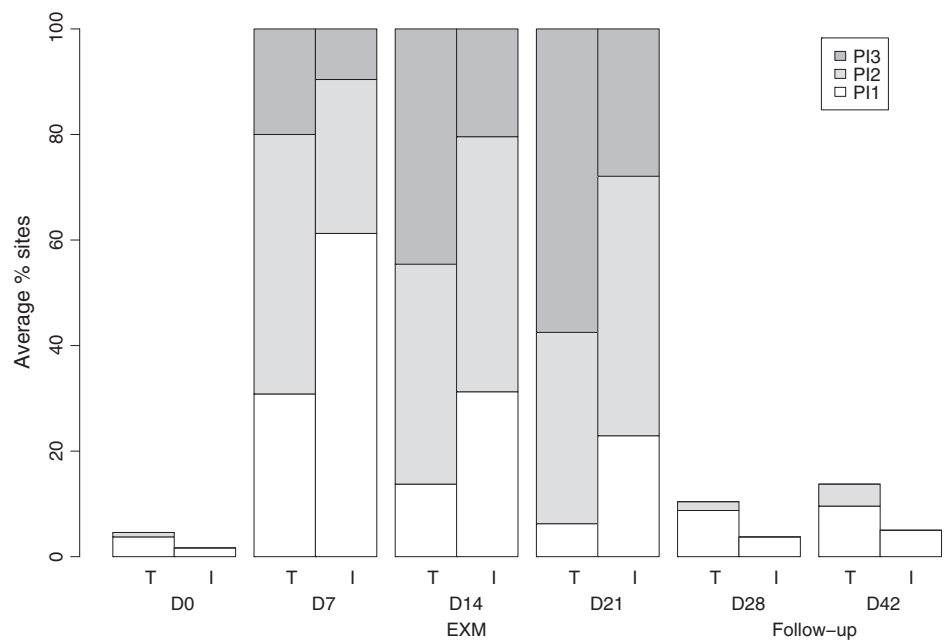


Fig. 2. Average proportion of sites with PI of 1, 2, and 3. EXM: experimental phase with no oral hygiene; T: teeth; I: implants.

were significantly higher in GCF compared to PCF at day 21. Some biomarkers decreased during the experimental phase: MIP-1 $\beta$  decreased significantly in GCF at day 7, 14, and 21, but not in PCF. GCF concentrations of MIP-1 $\beta$  were significantly lower than in PCF during the whole experimental phase. GM-CSF concentrations decreased significantly in GCF and PCF compared to baseline, and there was a significantly higher GM-CSF concentration in GCF than PCF on day 21. IL-8 decreased significantly in GCF with plaque accumulation on teeth.

In general, after re-establishing oral hygiene, the level of the different biomarkers had the tendency to return to the median concentrations found at baseline within

1 week. On day 28, PCF concentrations of GM-CSF and IFN- $\gamma$  were still significantly lower compared to baseline. In GCF, concentrations of IL-6, IL-17, GM-CSF, IFN- $\gamma$ , and MIP-1 $\beta$  were still significantly lower compared to baseline. On day 42, the levels of all biomarkers assessed in GCF and PCF had returned to values no longer different from those measured at day 0.

#### Discussion

The aim of this study was to compare the inflammation around dental implants and natural teeth during and after 3 weeks of undisturbed plaque accumulation in elderly

Table 3. Clinical parameters before (day 0), during (days 7, 14, 21) and after plaque accumulation (days 28, 42), expressed as median and interquartile range [IQR]. The GI is dichotomized as 0 or 1 vs. 2 or 3 (GI >1)

	Plaque accumulation			Oral hygiene		
	Day 0	Day 7	Day 14	Day 21	Day 28	Day 42
<b>Teeth</b>						
Mean PI	0.00 [0.00; 0.08]	<b>1.88</b> [1.65; 2.08]*	<b>2.42</b> [2.13; 2.58]*	<b>2.67</b> [2.31; 2.77]*	<b>0.08</b> [0.00; 0.17]*	<b>0.13</b> [0.00; 0.19]*
GI >1	0.00 [0.00; 0.00]	0.17 [0.17; 0.33]	0.42 [0.25; 0.60]*	<b>0.58</b> [0.42; 0.60]*	<b>0.00</b> [0.00; 0.02]	0.00 [0.00; 0.02]
Mean PD [mm]	<b>2.38</b> [2.23; 2.50]	–	–	<b>2.58</b> [2.31; 2.67]*	–	<b>2.25</b> [2.08; 2.44]
Mean BOP	0.00 [0.00; 0.02]	–	–	<b>0.58</b> [0.48; 0.67]*	–	0.04 [0.00; 0.08]
Mean REC [mm]	<b>1.13</b> [0.65; 1.71]	–	–	<b>1.21</b> [0.56; 1.52]	–	<b>1.25</b> [0.48; 1.90]
<b>Implants</b>						
Mean PI	0.00 [0.00; 0.00]	<b>1.33</b> [1.25; 1.52]*	<b>1.83</b> [1.42; 2.21]*	<b>2.00</b> [1.56; 2.44]*	<b>0.00</b> [0.00; 0.08]	<b>0.00</b> [0.00; 0.08]
GI >1	0.00 [0.00; 0.00]	0.25 [0.17; 0.42]	0.46 [0.31; 0.60]*	<b>0.63</b> [0.50; 0.75]*	<b>0.00</b> [0.00; 0.19]*	0.00 [0.00; 0.00]
Mean PD [mm]	<b>2.83</b> [2.73; 3.08]	–	–	<b>3.13</b> [2.88; 3.33]*	–	<b>2.75</b> [2.56; 3.00]
Mean BOP	0.00 [0.00; 0.10]	–	–	<b>0.71</b> [0.58; 0.85]*	–	0.00 [0.00; 0.17]
Mean REC [mm]	<b>0.29</b> [0.00; 0.81]	–	–	<b>0.33</b> [0.00; 0.85]	–	<b>0.21</b> [0.00; 0.77]

\*Significant difference when compared with value at day 0 ( $P < 0.05$ ).

Values in bold: significant difference between implants and teeth ( $P < 0.05$ ).



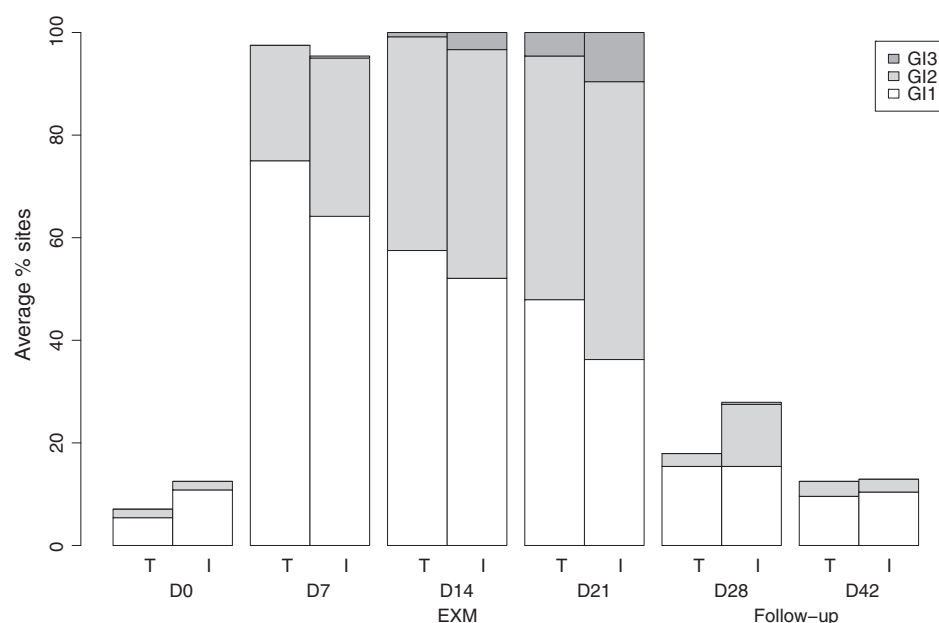


Fig. 3. Average proportion of sites with GI of 1, 2, and 3. EXM: experimental phase with no oral hygiene; T: teeth; I: implants.

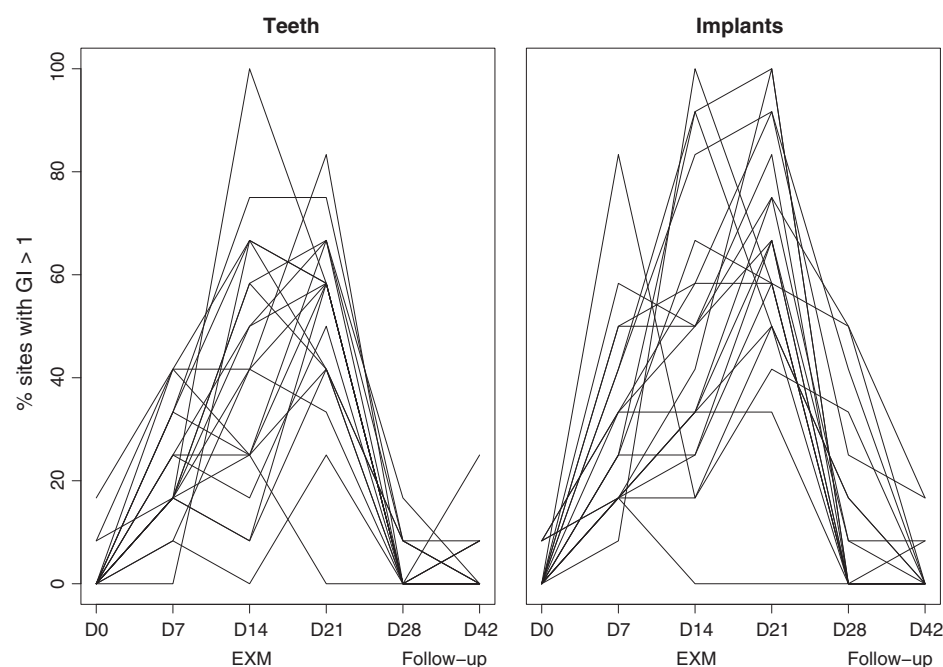


Fig. 4. Proportion of sites with GI > 1, from baseline to the end of follow-up time, for each participant. EXM: experimental phase with no oral hygiene.

persons. Millions of implants are placed worldwide every year. Given their high documented survival rates (Andriotelli et al. 2010; Pjetursson et al. 2012), these implants will still be *in situ* when the patients reach an old and very old age. Physiological aging implies a functional decline of vision, tactile sensitivity, and dexterity, rendering meticulous oral hygiene difficult. Furthermore, a shift in priorities may occur when chronic

diseases and functional impairment dominate daily life (Müller 2014). One of the inclusion criteria of this study was an age of 70 years or older. In most industrialized countries, the average life expectancy of men and women has now risen to over 80 years, and our experiments would have been even more relevant to a geriatric population if the minimum age had been around 80 years. However, recruiting in this age cohort proved

difficult, as most patients did either have implants, or natural teeth, but very rarely both, as required for the participation in this study. Furthermore, signs of periodontitis or peri-implantitis associated with poor oral hygiene precluded the enrollment of many otherwise eligible subjects. Nevertheless, with an average age of 77 years, the cohort in the present study is still substantially older than those from previous reports on experimental peri-implantitis and therefore provides a valuable and novel insight into the inflammatory tissue reactions in old age.

In agreement with previous studies, there was evidence for a cause-effect relationship between plaque accumulation and inflammation of both the peri-implant mucosa and the gingiva (Pontoriero et al. 1994; Zitzmann et al. 2001). Inflammation was clinically more pronounced around peri-implant tissues at the end of the experimental phase. After reintroduction of proper plaque control, all clinical parameters returned to pre-experimental values. This notion of reversibility was also in agreement with the literature (Löe et al. 1965; Salvi et al. 2012).

At baseline, PI, PD, and REC were significantly different between implants and teeth, with higher values of PD around implants and higher values of PI and REC around teeth. Implant restorations often present a less favorable “self-cleaning” morphology than natural teeth, due to their reduced diameter compared to a natural root as well as various other technical features, rendering oral hygiene measures more complex. Finding a lower PI on the implant sites seems therefore counterintuitive, but may be explained by the increased attention the patients may have attributed to their implants, for which they had undergone numerous treatment sessions and for which they have spent a substantial amount of money. A difference of 0.5 mm on PD between implants and natural teeth has been shown previously, thus confirming the present findings (Christensen et al. 1997). With the development of mucositis, PD further increased, in accordance with a previous study comparing implants with and without mucositis (Ata-Ali et al. 2013). As for REC, plaque accumulation did not show any significant effect in the experiments. This may be related to the short observation period, where in terms of recessions, an initial swelling of the gingiva may have compensated for the increased PD.

Our clinical results corroborate to those found by Salvi et al. (2012) who monitored clinical, microbiological, and host-derived

**Table 4.** Biomarkers before (day 0), during (days 7, 14, 21) and after plaque accumulation (days 28, 42). Concentrations are expressed in pg/ml and are median [IQR]

	Plaque accumulation			Oral hygiene		
	Day 0	Day 7	Day 14	Day 21	Day 28	Day 42
<b>Teeth</b>						
IL-1 $\beta$	87.3 [50.6; 115.1]	<b>247.4</b> [187.4; 443.2]*	<b>311.5</b> [151.3; 514.2]*	<b>375.1</b> [252.2; 679.1]*	103.7 [50.1; 205.1]	88.5 [41.4; 208.8]
IL-1ra	45.6 [26.8; 89.2]	108.2 [71.3; 163.1]*	68.6 [49.3; 111.5]	66.6 [35.4; 177.0]	61.3 [27.5; 408.6]	<b>36.0</b> [21.1; 51.1]
IL-6	<b>4.7</b> [1.3; 10.0]	1.8 [0.7; 2.6]*	1.7 [1.0; 3.0]*	1.1 [0.7; 2.5]*	1.3 [0.5; 3.1]*	2.6 [0.8; 4.3]
IL-8	429.3 [302.6; 528.2]	<b>242.2</b> [149.5; 472.0]*	<b>211.4</b> [144.4; 323.6]*	263.5 [194.9; 348.9]	299.2 [211.5; 478.4]	431.3 [219.0; 664.1]
IL-17	20.3 [14.3; 29.5]	18.9 [12.0; 29.2]	14.6 [0.0; 28.1]	13.3 [0.0; 30.0]	11.7 [0.0; 20.8]*	15.2 [6.8; 25.3]
Basic-FGF	31.7 [27.4; 35.4]	40.1 [32.3; 45.4]*	38.2 [1.7; 48.4]	30.8 [3.8; 39.1]	26.8 [0.0; 34.7]	30.1 [18.0; 41.3]
G-CSF	496.1 [107.3; 1115.9]	443.4 [170.3; 659.5]	236.4 [170.4; 433.7]	259.5 [67.7; 661.4]	273.3 [68.8; 596.3]	363.7 [29.0; 634.6]
GM-CSF	31.8 [20.6; 48.4]	27.3 [16.5; 41.0]*	25.0 [2.6; 40.0]	<b>18.5</b> [3.6; 41.1]*	26.7 [2.5; 39.9]*	30.1 [20.4; 42.3]
IFN- $\gamma$	123.2 [75.5; 140.0]	111.5 [103.0; 163.9]	106.1 [47.9; 148.8]	<b>92.3</b> [61.5; 120.2]	79.2 [15.8; 92.1]*	97.9 [52.9; 116.9]
MIP-1 $\beta$	17.8 [7.6; 27.8]	<b>5.3</b> [3.4; 8.5]*	4.7 [2.7; 6.6]*	<b>4.4</b> [3.1; 7.1]*	6.1 [4.3; 15.3]*	10.7 [7.9; 18.4]
TNF- $\alpha$	11.1 [6.4; 20.5]	12.8 [8.7; 19.4]	10.4 [6.3; 18.6]	<b>9.6</b> [5.8; 17.0]	7.2 [3.2; 11.6]	8.6 [5.7; 24.1]
VEGF	391.0 [260.8; 470.6]	339.7 [293.2; 558.2]	351.2 [284.0; 528.4]	317.8 [244.1; 725.0]	235.8 [138.1; 368.3]	340.8 [159.3; 474.3]
<b>Implants</b>						
IL-1 $\beta$	80.9 [38.0; 146.0]	<b>161</b> [76.3; 331]*	<b>166.8</b> [116.6; 249.4]*	<b>163.4</b> [81.0; 288.0]*	81.7 [44.2; 132.2]	86.1 [62.5; 228.2]
IL-1ra	61.5 [31.5; 213.5]	125.1 [36.7; 186]*	103.0 [50.8; 179.7]	54.2 [33.6; 119.6]	75.7 [39.1; 104.6]	<b>44.9</b> [27.5; 73.3]
IL-6	<b>1.9</b> [1.0; 5.6]	1.8 [0.83; 2.74]	1.3 [0.5; 2.4]*	1.6 [0.3; 2.4]	1.3 [0.9; 2.1]	1.8 [0.5; 6.6]
IL-8	419.8 [232.6; 904.3]	<b>392</b> [224; 878]	<b>394.4</b> [222.5; 486.8]	231.3 [168.8; 551.9]	359.3 [220.6; 523.1]	498.6 [184.6; 823.5]
IL-17	19.4 [12.6; 28.9]	22.1 [9.44; 32.7]	11.3 [0.0; 30.7]	3.5 [0.0; 20.6]	11.8 [0.0; 25.3]	14.2 [1.5; 23.6]
Basic-FGF	31.3 [27.0; 35.3]	37.7 [28.9; 44]	34.7 [0.01; 52.4]	19.2 [0.3; 35.7]	22.0 [0.0; 40.6]	34.0 [13.9; 36.5]
G-CSF	257.3 [149.4; 667.0]	263 [108; 561]	192.5 [41.0; 404.3]	65.9 [9.1; 259.8]*	35.9 [8.9; 395.9]	220.5 [18.3; 432.4]
GM-CSF	34.2 [20.7; 47.3]	25.9 [15; 36.5]*	17.2 [2.2; 39.2]*	<b>7.9</b> [1.1; 35.6]*	21.0 [2.8; 39.5]*	30.7 [12.3; 40.5]
IFN- $\gamma$	92.4 [80.7; 129.3]	119 [68.8; 143]	89.4 [26.8; 140.4]	<b>56.6</b> [25.1; 88.1]	54.5 [17.1; 104.3]*	92.6 [26.0; 126.4]
MIP-1 $\beta$	12.6 [7.1; 26.8]	<b>11.9</b> [6.84; 21.5]	7.5 [4.8; 19.2]	<b>8.3</b> [3.9; 26.1]	12.3 [6.1; 24.1]	12.1 [8.1; 29.4]
TNF- $\alpha$	7.1 [5.7; 17.8]	11.8 [8.12; 17.5]	9.4 [2.7; 13.2]	<b>8.7</b> [2.3; 12.3]	7.6 [3.6; 15.8]	14.6 [3.0; 27.6]
VEGF	400.2 [211.1; 564.8]	368 [212; 702]	396.1 [218.2; 645.3]	328.0 [156.8; 506.5]	157.1 [127.6; 374.9]	303.6 [141.6; 444.6]

\*Significant difference when compared with value at day 0 ( $P < 0.05$ ).Values in bold: significant difference between implants and teeth ( $P < 0.05$ ).

alterations around teeth and titanium implants during the development of experimental gingivitis/mucositis: less plaque accumulation but more inflammation developed around implants compared to teeth. However, 3 weeks of resumed plaque control did not yield pre-experimental levels of gingival inflammation around implants in their study, whereas in our trial, inflammation returned to baseline levels around both implants and teeth.

We compared the inflammatory response during a phase of undisturbed plaque accumulation at implants and teeth by analysis of levels of 12 cytokines in the GCF and PCF. At baseline, no significant differences were observed in the expression of any cytokine in GCF and PCF. This is in accordance with previous studies that showed that under healthy clinical conditions, the expression of biomarkers does not differ between implants and teeth (Tsalikis 2010; Salvi et al. 2012; Cionca et al. 2016; Ramseier et al. 2016). With the development of inflammation, significant changes were observed for several biomarkers assessed around implants and teeth that returned to baseline levels by the end of the experiment. The association between signs of inflammation and the expression of biochemical markers in GCF and PCF has been shown previously

(Offenbacher et al. 2010; Petkovic et al. 2010; Guncu et al. 2012; Ramseier et al. 2016).

During the development of gingivitis/mucositis, the most significant difference between implants and teeth was found for IL-1 $\beta$ , with higher levels obtained around teeth. Smaller differences were observed for some other markers, such as IL-8, GM-CSF, IFN- $\gamma$ , MIP-1 $\beta$ , and TNF- $\alpha$ . A recent cross-sectional study in a population with a mean age of  $71 \pm 9$  years reported that IL-1 $\beta$  and IL-8 had a tendency to be lower in a mucositis group, when compared to healthy controls (Hall et al. 2015). Compared to our study, contradictory results were obtained by another study (Salvi et al. 2012), which found no significant differences on the expression of IL-1 $\beta$  between implants and teeth with the development of inflammation. Although the experimental model used in both studies was similar, the only difference that could explain these discrepancies is the age of the population: their study included subjects between 28 and 75 years old (Salvi et al. 2012), whereas in our study, all participants were at least 70 years old. Experimental gingivitis is comparable but not identical to chronic gingivitis. As previously reported (Deinzer et al. 2007), variations in immunological parameters over 4 weeks of experimental

gingivitis were considerable, whereas only small fluctuations were observed with chronic gingivitis. Twenty-one days of experimental gingivitis/mucositis may not be long enough to discriminate permanent differences in cytokine profiles around implants and teeth.

The influence of age on gingival health has been investigated in several clinical trials. Some indicated that gingivitis develops more quickly and is more pronounced in older persons as compared to younger ones (Holm-Pedersen et al. 1975; Van der Velden 1984; Fransson et al. 1996). Others found no impact of age on gingival inflammation. For example, GCF flow in young and older subjects increased similarly during inflammation (Borden et al. 1977). Another experimental gingivitis trial showed that age had an effect on clinical parameters such as plaque and bleeding scores, but had no impact on GCF levels of studied cytokines; similar levels were expressed in younger and older persons (Tsalikis 2010). When interpreting these findings, it must be born in mind that the elderly dentition more often presents with niches due to recession of papillae and gingiva and that the prevalence of dental restorations is also higher in older age cohorts. Crown margins, denture clasps and both, fixed and removable replaced teeth all facilitate plaque adhesion,

thus explaining the increased plaque and bleeding scores.

Immunosenescence renders elderly individuals more prone to infections, but these are less often acute than in younger persons. A review (Hajishengallis 2010) showed that phagocytosis declines with age, thus emphasizing that age affects the immune system. On the whole, even if there are limited numbers of studies dealing with the effect of age on neutrophils, it seems that their function and capacity to chemotaxis can be affected as well (Butcher et al. 2000; Scott & Krauss 2012).

In summary, the present study has shown in persons aged 70 or over that plaque accumulation induces an inflammatory reaction around both teeth and implants. Although there was less plaque accumulation on implants, the peri-implant mucosa showed a

stronger clinical response than gingiva. A cause-and-effect relationship was confirmed. On a biomarker level, IL-1 $\beta$  was found to increase significantly around both implants and teeth. The significantly higher expression of IL-1 $\beta$ , TNF- $\alpha$ , IFN- $\gamma$ , and GM-CSF around teeth contrasted the higher GI scores at implants. Experimental gingivitis and mucositis were reversible both clinically and biochemically.

It can therefore be concluded that meticulous oral hygiene remains important in old age, for both, natural teeth and implant restorations. The reported higher susceptibility of the peri-implant tissues to signs and symptoms of inflammation compared to the periodontal tissues of natural teeth requires an even tighter and lifelong recall regimen to assure oral hygiene and peri-implant health.

**Acknowledgements:** The study was financed by research funds of the Division of Periodontology, University of Geneva School of Dental Medicine, Geneva, and was supported in part by an unrestricted research grant from the Swiss Society of Periodontology. The help of Jose Cancela (Division of Periodontology) in the biochemical analysis of GCF and PCF, and the contribution of Murali Srinivasan (Division of Gerodontology and Removable Prosthodontics) in editing the manuscript are greatly appreciated.

## Conflict of interest

The authors report no conflict of interests related to this study.

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